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Molecular determinants of humoral immune specificity for the occupational allergen, methylene diphenyl diisocyanate

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Abstract

Methylene diphenyl diisocyanate (MDI), a low molecular weight chemical important for producing polyurethane foam, coatings, and elastomers is a major cause of occupational asthma, however, mechanisms of disease pathogenesis remain poorly understood. This study characterizes the rearranged germline and hypervariable region cDNA of new anti-MDI secreting hybridomas derived from mice immunized with MDI-conjugated to autologous serum proteins. Six IgG1 secreting clones were identified in initial screening ELISAs, based on differential binding to MDI conjugated human albumin vs. mock exposed albumin. The mAbs secreted by the hybridomas also recognized MDI conjugated to other model proteins (e.g. ovalbumin, transferrin), but did not bind unconjugated proteins, or protein conjugates prepared with other isocyanates (e.g. TDI, HDI). The mAbs displayed (MDI)-dose dependent binding in ELISA and Western blot, and exhibited varying degrees of cross-competition, suggesting differences in epitope specificity. The cDNA encoding the monoclonal antibodies reveal clonal differences in the CDR3 regions, germline gene usage, and patterns of somatic hypermutation related to epitope specificity. Together, the data provide new insight into the molecular determinants of humoral MDI specificity, and characterize anti-MDI IgG1 secreting mAbs that may be developed into useful diagnostic reagents.

Keywords

methylene diphenyl diisocyanate (MDI); albumin; monoclonal antibody (mAb); polyurethane

INTRODUCTION

Methylene diphenyl diisocyanate (MDI) is the most abundantly produced and consumed diisocyanate, with applications in the production of truck-bed liners and spray foam insulation, among other uses (Allport et al., 2003). Like other isocyanates, MDI possesses the potential to sensitize the immune system, and is a well-recognized cause of occupational asthma world-wide (Chang and Karol, 1984; Lesage et al., 2007; Lofgren et al., 2003).

The mechanisms that mediate MDI asthma pathogenesis have been challenging to define, in part due to uncertainty regarding the antigenic form of the chemical *in vivo* (Wisnewski et

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

al., 2010; Wisnewski et al., 2000; Wisnewski et al., 2004). Recent studies in mice highlight the potential for isocyanate skin exposure to induce immune sensitization and hyper-responsiveness to subsequent respiratory tract exposure, and identify albumin as an important carrier protein for this process (Ban et al., 2006; Tarkowski et al., 2007; Wisnewski et al., 2011). In humans, albumin is the only carrier protein known to support antibody recognition of isocyanate (Liu and Wisnewski, 2003; Wass and Belin, 1989; Wisnewski et al., 2004). IgE that binds isocyanate-albumin conjugates, sometimes develops in association with isocyanate asthma, and exposure is commonly associated with IgG responses (Aul et al., 1999; Jin and Karol, 1988; Wisnewski et al., 2010).

In this study, we developed hybridomas that secrete anti-MDI IgG1 and analyzed their cDNA, to begin defining rearranged germline and hypervariable antibody (heavy and light chain) gene combinations that encode MDI recognition. The specificities of the newly developed mAbs are tested in ELISA and Western blots with MDI-conjugated proteins and unconjugated controls, with a focus on human albumin, as a clinically relevant carrier protein. The distinct molecular characteristics of the anti-MDI mAbs are discussed in relation to their relative binding specificity, and future diagnostic or research use.

2. Materials and Methods

2a. Reagents

Complete and Incomplete Freund's Adjuvant were from Thermo Fisher Scientific (Rochester, NY). 4, 4'-Methylenebis(phenylisocyanate) (MDI), toluene-2,4-diisocyanate (TDI), and 1,6-hexamethylene diisocyanate, were from Aldrich (St Louis, MO). Acetone and HCl were from JT Baker (Phillipsburg, NJ) and Balb/C mouse serum was from Innovative research (Novi, MI). Human and mouse albumin, turkey egg ovalbumin, FITC-BSA, human transferrin, and 50% (w/v) polyethylene glycol were from Sigma (St. Louis, MO). Dry milk was from American Bioanalytical (Natick, MA).

2b. Vertebrate Animals

Female Balb/C mice were obtained from Jackson Laboratories (Barr Harbor, ME). All animal studies were approved by the Yale Animal Care Committee, according to IACUC rules and regulations regarding animal research. Mice were immunized intraperitoneally (ip) with 100 μ g of mouse serum protein, which had been conjugated with MDI (see below), emulsified in complete Freund's adjuvant. Mice were rested for 3 weeks and then given two booster shots (100 μ g) ip with incomplete Freund's adjuvant, three weeks apart, followed by a final intravenous booster (50 μ g) in PBS four days before obtaining splenocytes.

2b. MDI-conjugated proteins

MDI (w/v) prediluted in acetone to 10% (w/v), was mixed with serum (diluted 1:5 in PBS) or purified protein solutions (human albumin, transferrin and ovalbumin) in PBS (5 mg/ml) to achieve a final MDI concentration of 0.1% (w/v) or 4 mM. Following 2 h reaction at 37°C with mixing, samples were microfuged at $8,000 \times g$ and 0.2 μ M filtered, and dialyzed 4X vs. PBS. Samples were aliquoted and stored at -80°C until needed. Control samples of unconjugated proteins were similarly mock exposed to acetone without MDI, and identically processed. In some experiments, human albumin was exposed to lower doses of MDI (e.g. 0.01%, and 0.001% w/v) under identical reaction conditions, to obtain MDI-albumin conjugates with lower amounts of MDI per albumin molecule.

2c. Somatic cell hybridization

The spleens of mice immunized with MDI-conjugated serum proteins were ground between the ends of autoclaved, frosted glass slides, to obtain a cellular suspension in Hanks

Balanced Basic Saline Solution (HBBS) (GibcoBRL; Grand Island, NY). Following lysis of RBCs with NH_4Cl , splenocytes were fused with 2×10^7 SP20 cells (ATTC; Manassas, VA) using 50% (w/v) PEG solution. (Wiest et al., 1991) Immediately after fusion, cells were rested in complete culture media with IL-6 for 6 h, before seeding into 96-well plates in media supplemented with HT. Selective media containing aminopterin was added 24-h later. Following identification of positive hybridomas, cultures were expanded and subcloned by limiting dilution.

2d. ELISA screening for anti-MDI IgG mAbs

Culture supernatant of hybridomas were initially screened by ELISA for murine IgG that bound MDI conjugated human albumin, based on prior conditions established in prior reports (Wisnewski et al., 2010; Wisnewski et al., 2004; Wisnewski et al., 2012; Wisnewski et al., 2011; Ye et al., 2006). In later studies, specificity was analyzed in ELISAs against mock exposed human albumin, ovalbumin, transferrin, MDI-ovalbumin, MDI-transferrin, and FITC-BSA (Sigma). Nunc Maxisorp microtiter plates, obtained through Thermo Fisher Scientific, were coated with 100 μl /well of the antigen, diluted to 10 $\mu\text{g}/\text{ml}$ in 0.1 M carbonate buffer (pH 9.5), by overnight incubation at 4°C. Plates were blocked with 3% (w/v) dry milk before addition of culture supernatant, neat or diluted in PBS 1:4. Supernatants was incubated for 1 hour at 25°C, followed by a 1:2000 dilution of peroxidase conjugated goat anti-murine IgG F_c -specific from BD Pharmingen (San Diego, CA). ELISAs were developed for up to 40 min with TMB substrate from BD Bioscience (San Jose, CA); and the final reactions were terminated by addition of 0.1 M HCl from Sigma. Optical density (O.D.) measurements were obtained on a Benchmark microtiter plate reader from Bio-Rad (Hercules, CA), using dual wavelength absorbance (450–550 nm). All experiments were performed three times, sample permitting, to obtain mean values expressed in figures.

2e. Electrophoresis

Albumin and MDI-albumin samples were mixed with commercial sample buffers (2X) from Bio-Rad, for reducing, non-reducing, and native gel electrophoresis. Reducing and non-reducing buffers contained 62.5mM Tris (pH 6.8), 40% (v/v) glycerol and 0.01% (w/v) bromophenol blue. Reducing buffer also contained 5% (v/v) 2-mercaptoethanol. Sodium dodecyl sulfate (SDS) acrylamide gels (4–15% gradient) were obtained pre-cast from BioRad. Native gels were prepared using a commercial acrylamide mixture from American Bioanalytical (Natick, MA), which contained 30.8% (w/v) acrylamide/bis (37.5:1). Electrophoresis was performed as previously described, and gels were stained with commercial coomassie-based buffer Imperial Protein stain from Pierce (Rockford, IL).

2f. Western blot

Polyacrylamide gels were transferred to nitrocellulose membrane using a transblot system from BioRad. Nitrocellulose membranes were blocked with 3% (w/v) dry milk in PBS, and probed with hybridoma culture supernatant. Following incubation with 1:1000 peroxidase-conjugated anti-mouse IgG (Pharmingen; San Diego, CA), blots were developed with enhanced luminescence reagent from Thermo Fisher Scientific.

2g. Isotype analysis

The isotype of the mouse mAbs was determined using a Pierce's Rapid ELISA Mouse mAb Typing Kit, from ThermoFisher Scientific.

2h. Purification and biotinylation of mAbs

Culture supernatant was precipitated with 40% (w/v) ammonium sulfate, and the pellet was redissolved in PBS and dialyzed extensively. The IgG fraction was purified using Protein G

from GE Healthcare (Pittsburgh, PA), and quantitated using a commercial Protein assay from Bio-Rad (Hercules, CA), with murine IgG as the reference standard. Purified mAbs were labeled with biotin, using no-weigh sulfo-NHS-biotin kit from Pierce/Thermo Fisher Scientific.

2j. Cross-competition ELISAs

Competitive ELISAs were performed using plates coated with MDI-albumin as described above, with the following modifications. Unlabeled mAb at 0, 5, or 50 µg/ml was added to wells for 1 hr, and then biotinylated mAb AD3 or AH6 was additionally added for another hour. ELISAs were developed with streptavidin-HRP and % inhibition of biotinylated mAb binding was calculated based on OD values. Biotinylated mAbs were used at concentrations within the linear range of detection, based on preliminary ELISAs. All samples were tested in triplicate.

2k. Amplification and sequencing of mAb genes

Hybridoma mRNA was purified using oligotex direct mRNA mini columns from Qiagen, and used as the template for switch mechanism activated reverse transcriptase from Invitrogen, in order to generate cDNA capped on the 5' end, with sequence complementary to a universal primer. The cDNA was used in touchdown PCR with primers specific for murine IgG1 (5'-CTCAATTTTCTTGTCACCTTGGTGC-3'), or kappa constant regions (5'-CTCATTCCTGTTGAAGCTCTTGAC-3'), to amplify the heavy and light chain variable regions, via a 5'-RACE like reaction. PCR products were gel purified using SNAP columns, and directly sequenced using sequencing primers specific for the 5' end of IgG1 (5'-GTTAGTTTGGGCAGCAGA-3') or kappa constant region (5'-GACTGAGGCACCTCCAGA-3'). Sequencing was performed by the Yale Keck Center, and alignment with germline genes was performed using on-line software from IMGT. The following mAb heavy and light chain sequences have been submitted to GeneBank under the following accession numbers: AD3VH (JX982622), AD3VL (JX982623), AH6VH (JX982624), AH6VL (JX982625), BC8VH (JX982633), BC8VL (JX982632), CE2VH (JX982626), CE2VL (JX982627), DA5 VH (JX982628), DA5VL (JX982629), DD10VH (JX982630), DD10VL (JX982631).

3. Results

3a. Identification of hybridomas secreting anti-MDI IgG mAbs

ELISA screening of supernatants from hybridoma cultures identified six clones that secrete IgG which bound to MDI conjugated proteins (human albumin and transferrin, mouse albumin, and turkey egg ovalbumin), but did not bind other isocyanate (HDI or TDI) conjugated proteins, or mock exposed (unconjugated) proteins (Figure 1). Little or no cross-reactivity was observed with the closely related chemical (fluorescein) isothiocyanate. Anti-MDI secreting hybridomas were subcloned by limiting dilution, and determined to produce IgG1/kappa mAbs.

3b. Western blot analysis and MDI-dose dependent binding of mAbs

Western blot studies were performed to further evaluate the new mAbs specificity, including their ability to recognize MDI-protein conjugates denatured by SDS and reducing agent. As shown (Fig. 2A), the different anti-MDI mAbs recognize MDI-albumin, but not unconjugated albumin, in Western blots. The observed triple banding pattern is consistent with previous studies demonstrating monomeric, dimerized and trimeric reaction products of MDI-albumin resulting from *in vitro* conjugation (Aul et al., 1999; Jin and Karol, 1988), and the mAbs ability to recognize MDI in all three reaction products. In other Western blots, the

MDI-dose dependence of mAb binding was evaluated. MDI-albumin conjugates prepared with different starting amounts of MDI (ranging from 0.001% to 0.1% w/v) exhibited mAb binding levels proportional to MDI conjugation per albumin molecule (Fig. 2B and not shown).

3c. Cross-competition studies

To begin evaluating the epitope specificity of the mAbs, relative to one another, competitive binding studies were performed. The ability of increasing concentrations of unlabeled mAb, to inhibit the binding of another biotinylated mAb (to MDI-albumin), was determined by ELISA. As shown in Fig 3, mAbs BC8 and CE2 appear to possess epitope specificities distinct from mAbs AD3 and AH6, as concentrations up to 20 µg/ml of the respective unlabeled mAbs cause only minimal inhibition of the respective biotinylated mAb AD3 or AH6. In contrast, unlabeled mAbs AH6 and AD3 compete similarly with the labeled mAbs suggesting these different mAbs recognize either similar or overlapping epitopes.

3d. Molecular analysis of new anti-MDI IgG1 mAbs

The variable regions of the heavy and light chain genes, which encode the new anti-MDI mAbs, were amplified using a 5'-RACE like approach as described in the Methods section 2k. Comparison of the amplified DNA sequences with the *Mus musculus* genome identified the most likely germline genes from which the antibodies were derived, as well as the sequence of the rearranged hypervariable regions.

Overall, the mAb heavy chain genes exhibited diversity in CDR3 length and amino acids, and changes in V-region sequence (compared with their most likely germline genes) indicative of affinity maturation. The mAb BC8 possesses perhaps the most distinctive VH CDR3, 10 amino acids in length, with an unusual stretch of four threonines in a row, while mAb CE2 exhibits the most unique combination of germline V, D and J-region genes. The remaining four mAbs each possessed longer CDR3s (13 or 14 amino acids) and used heavy chain V-regions from family V5, according to IMGT numbering (Lefranc, 2003), the largest murine VH family, also known as the VH7183 family (Brodeur and Riblet, 1984). The mAb CE2's heavy chain uses germline gene V6-6*01, a member of the VH family previously named J606, whose members contain a binding site for protein A, distinct from the Fc region, an important characteristic that influences purification (Brodeur and Riblet, 1984; Ibrahim et al., 1993; Seppala et al., 1990). The mAb BC8's heavy chain appears to be derived from V3-2*02, which belongs to the VH 36–60 family, known to encode serologically-defined idiotypes elicited by other low molecular weight chemicals, particularly p-azophenylarsonate (Brodeur and Riblet, 1984; Juszczak et al., 1984; Near et al., 1984).

The genes for the mAb's light chains possessed more limited diversity in their CDR3-regions and relatively fewer nucleotide changes vs. V and J-region germline genes, compared with the heavy chains. All six of the mAbs contained 9 amino acids in their CDR3, with Q, P and T at positions 2, 7, 9. Three of the mAbs, which possessed nearly identical VH CDR3s, also possessed nearly identical VL CDR3s, and contained V and J-regions with little or no change compared to the germline configuration.

The molecular characteristics of the new anti-MDI mAbs are consistent with competitive binding studies suggesting differences in epitope specificity. The mAbs BC8 and CE2, which fail to effectively compete with mAbs AD3 or AH6, for binding to MDI-albumin, possesses distinct VH and VL CDR3 and germline gene sequences. In contrast, mAbs AH6 and AD3, with overlapping epitope specificity based on cross-competition studies, express nearly the same VH and VL CDR3 and were apparently derived from the same, or highly

related germline genes. Together, the data identify combinations of specific germline genes and hypervariable region sequences that encode for immune recognition of the occupational allergen, MDI.

4. DISCUSSION

This study is the first to develop IgG monoclonal antibodies that recognize the low-molecular weight chemical MDI, a crucial starting material for making polyurethane, and well-recognized cause of occupational asthma world-wide. Hybridomas secreting the anti-MDI mAbs were derived from mice immunized with self (serum) proteins, which had been conjugated with MDI *ex vivo*. Molecular characterization of the hybridomas' rearranged cDNA identified clonally distinct antibody heavy and light chain combinations that encode MDI recognition. Competitive binding studies identified differences in mAb's epitope specificity, which were associated with amino acid sequence of the CDR3 region and germline gene usage. Together, the data provide insight into molecular determinants of MDI (humoral) immune specificity, and define the DNA sequences for murine mAbs, with potential use as future diagnostic/research reagents.

The 6 new anti-MDI mAbs are each unique, however, several possess identical or nearly identical amino acid sequences in the CDR3 region of their heavy and/or light chains, suggesting their selective expansion in response to MDI. All of the light chain CDR3s are 9 amino acids in length, and contain Q, P and T at CDR3 positions 2, 7, 9. In contrast, the heavy chains have CDR3s more variable in length (10–14 amino acids) and codon usage, and exhibit molecular evidence of having undergone an affinity maturation process, based on amino acid changes compared with germline CDR1/CDR2 and J-regions. The heavy chain CDR3s for three of the mAbs are highly homologous, and were likely derived from identical germline VH and J-regions, and either identical or highly similar D-region genes. However, the heavy chains of the other 3 anti-MDI mAbs are relatively unique, with distinct CDR3 amino acid sequences, V, D, and J region usage.

One of the six new anti-MDI mAbs, BC8 possesses perhaps the most interesting molecular properties, including 4 consecutive threonines in the heavy chain CDR3, and usage of germline V3-2*02 (by IMGT numbering). The V3-2*02 gene is also known as VH 36–60, and has been well studied, as it encodes a serologically-defined idiotype associated with exposure to certain low molecular weight chemical haptens, especially p-azophenylarsonate (Juszczak et al., 1984; Near et al., 1984; Pincus, 1988). The human VH genes most homologous to murine VH36–60 belonging to the small VH4 gene family (Pincus, 1988), frequently associated with autoantibodies (Stevenson et al., 1995), which may be relevant to isocyanate exposure, given that the self protein albumin is the major carrier protein for isocyanate *in vivo*, and the reported increase of anti-keratin antibodies in isocyanate asthma patients (Choi et al., 2004).

The anti-MDI IgG1 mAbs described here may form the basis of assays for future diagnostic or research purposes; for example, to quantitate MDI levels in blood or urine, or to standardize MDI antigens for serology. The mAbs might be used to identify MDI *in vitro* in tissue culture, or *in vivo* in animal models of exposure. The mAb genes could be molecularly modified to further investigate the role of different CDR3 amino acids, germline V-region, and constant region isotypes on MDI specificity and effector activity. The present molecular data elucidating the cDNA sequence of the newly generated anti-MDI mAbs, will facilitate their future development as diagnostic and/or basic research reagents.

In summary, hybridomas secreting IgG1 mAbs that recognize the occupational allergen, MDI, were developed and molecularly analyzed to identify critical determinants of chemical

recognition by the immune system. The data provide important information towards development of new immunology-based approaches to exposure surveillance, harnessing the specificity and sensitivity of mAbs to detect and quantitate MDI present in human tissue or environmental samples.

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ABBREVIATIONS

D	diversity
FITC	fluorescein isothiocyanate
HDI	hexamethylene diisocyanate
J	junction
MDI	methylenediphenyl diisocyanate
TDI	toluene diisocyanate
V	variable
VH	variable portion of the heavy chain
VL	variable portion of the light chain

REFERENCES

- Allport, DC.; Gilbert, DS.; Outterside, SM. MDI and TDI : safety, health and the environment : a source book and practical guide. Chichester: Wiley; 2003.
- Aul DJ, Bhaumik A, Kennedy AL, Brown WE, Lesage J, Malo JL. Specific IgG response to monomeric and polymeric diphenylmethane diisocyanate conjugates in subjects with respiratory reactions to isocyanates. *J Allergy Clin Immunol.* 1999; 103:749–755. [PubMed: 10329805]
- Ban M, Morel G, Langonne I, Huguet N, Pepin E, Binet S. TDI can induce respiratory allergy with Th2-dominated response in mice. *Toxicology.* 2006; 218:39–47. [PubMed: 16271432]
- Brodeur PH, Riblet R. The immunoglobulin heavy chain variable region (Igh-V) locus in the mouse. I. One hundred Igh-V genes comprise seven families of homologous genes. *European journal of immunology.* 1984; 14:922–930. [PubMed: 6092095]
- Chang KC, Karol MH. Diphenylmethane diisocyanate (MDI)-induced asthma: evaluation of the immunologic responses and application of an animal model of isocyanate sensitivity. *Clinical allergy.* 1984; 14:329–339. [PubMed: 6467557]
- Choi JH, Nahm D, Kim SH, Kim YS, Suh CH, Park HS, Ahn SW. Increased levels of IgG to cytokeratin 19 in sera of patients with toluene diisocyanate-induced asthma. *Annals of Allergy, Asthma & Immunology.* 2004; 93:293–298.
- Ibrahim S, Kaartinen M, Seppala I, Matoso-Ferreira A, Makela O. The alternative binding site for protein A in the Fab fragment of immunoglobulins. *Scandinavian journal of immunology.* 1993; 37:257–264. [PubMed: 8434237]
- Jin RZ, Karol MH. Intra- and intermolecular reactions of 4,4'-diisocyanatodiphenylmethane with human serum albumin. *Chem Res Toxicol.* 1988; 1:281–287. [PubMed: 2979744]
- Juszczak E, Near RI, Geffer ML, Margolies MN. Complete heavy and light chain variable region sequence of anti-arsenate monoclonal antibodies from BALB/c and A/J mice sharing the 36–60 idiotype are highly homologous. *J Immunol.* 1984; 133:2603–2609. [PubMed: 6207237]

- Lefranc MP. IMGT, the international ImMunoGeneTics database. *Nucleic acids research*. 2003; 31:307–310. [PubMed: 12520009]
- Lesage J, Stanley J, Karoly WJ, Lichtenberg FW. Airborne methylene diphenyl diisocyanate (MDI) concentrations associated with the application of polyurethane spray foam in residential construction. *Journal of occupational and environmental hygiene*. 2007; 4:145–155. [PubMed: 17249149]
- Liu Q, Wisnewski AV. Recent developments in diisocyanate asthma. *Ann Allergy Asthma Immunol*. 2003; 90:35–41. [PubMed: 12772950]
- Lofgren DJ, Walley TL, Peters PM, Weis ML. MDI Exposure for Spray-On Truck Bed Lining. *Applied occupational and environmental hygiene*. 2003; 18:772–779. [PubMed: 12959888]
- Near RI, Juszczak EC, Huang SY, Sicari SA, Margolies MN, Gefter ML. Expression and rearrangement of homologous immunoglobulin VH genes in two mouse strains. *Proceedings of the National Academy of Sciences of the United States of America*. 1984; 81:2167–2171. [PubMed: 6326109]
- Pincus SH. Human immunoglobulin heavy-chain-variable (VH) region gene families defined by hybridization with cloned human and murine VH-genes. *Human immunology*. 1988; 22:199–215. [PubMed: 2902053]
- Seppala I, Kaartinen M, Ibrahim S, Makela O. Mouse Ig coded by VH families S107 or J606 bind to protein A. *J Immunol*. 1990; 145:2989–2993. [PubMed: 2120342]
- Stevenson FK, Spellerberg MB, Chapman CJ, Hamblin TJ. Differential usage of an autoantibody-associated VH gene, VH4–21, by human B-cell tumors. *Leukemia & lymphoma*. 1995; 16:379–384. [PubMed: 7787747]
- Tarkowski M, Vanoirbeek JA, Vanhooren HM, De Vooght V, Mercier CM, Ceuppens J, Nemery B, Hoet PH. Immunological determinants of ventilatory changes induced in mice by dermal sensitization and respiratory challenge with toluene diisocyanate. *American journal of physiology. Lung cellular and molecular physiology*. 2007; 292:L207–L214. [PubMed: 16963530]
- Wass U, Belin L. Immunologic specificity of isocyanate-induced IgE antibodies in serum from 10 sensitized workers. *J Allergy Clin Immunol*. 1989; 83:126–135. [PubMed: 2536411]
- Wiest PM, Wisnewski AV, Johnson JH, Ramirez B, Kresina TF, Olds GR. Screening of murine monoclonal antibodies against living schistosomula of *Schistosoma mansoni* by radioimmunoassay. *Int J Parasitol*. 1991; 21:449–454. [PubMed: 1917285]
- Wisnewski AV, Liu J, Redlich CA. Antigenic changes in human albumin caused by reactivity with the occupational allergen diphenylmethane diisocyanate. *Anal Biochem*. 2010; 400:251–258. [PubMed: 20123080]
- Wisnewski AV, Srivastava R, Herick C, Xu L, Lemus R, Cain H, Magoski NM, Karol MH, Bottomly K, Redlich CA. Identification of human lung and skin proteins conjugated with hexamethylene diisocyanate in vitro and in vivo. *Am J Respir Crit Care Med*. 2000; 162:2330–2336. [PubMed: 11112159]
- Wisnewski AV, Stowe MH, Cartier A, Liu Q, Liu J, Chen L, Redlich CA. Isocyanate vapor-induced antigenicity of human albumin. *J Allergy Clin Immunol*. 2004; 113:1178–1184. [PubMed: 15208602]
- Wisnewski AV, Stowe MH, Nerlinger A, Opare-Addo P, Decamp D, Kleinsmith CR, Redlich CA. Biomonitoring Hexamethylene Diisocyanate (HDI) Exposure Based on Serum Levels of HDI-Specific IgG. *Ann Occup Hyg*. 2012
- Wisnewski AV, Xu L, Robinson E, Liu J, Redlich CA, Herrick CA. Immune sensitization to methylene diphenyl diisocyanate (MDI) resulting from skin exposure: albumin as a carrier protein connecting skin exposure to subsequent respiratory responses. *J Occup Med Toxicol*. 2011; 6:6. [PubMed: 21414210]
- Ye YM, Kim CW, Kim HR, Kim HM, Suh CH, Nahm DH, Park HS, Redlich CA, Wisnewski AV. Biophysical determinants of toluene diisocyanate antigenicity associated with exposure and asthma. *J Allergy Clin Immunol*. 2006; 118:885–891. [PubMed: 17030242]

HIGHLIGHTS

- 1st IgG mAbs made against occupational allergen methylene-diphenyl-diisocyanate (MDI)
- cDNA of anti-MDI mAbs reveal clonally distinct origins
- Homology in CDR3 suggests antigen-driven selection for certain amino acids
- New anti-MDI mAbs should be useful diagnostic reagents for biomonitoring MDI exposure

Figure 1.

New murine monoclonal antibodies specific binding to MDI-conjugated proteins. ELISA data demonstrate the binding (O.D. value on Y-axis) of different mAbs (X-axis) to unconjugated control mock exposed proteins, MDI, HDI, TDI or FITC conjugated proteins, as labeled. Alb= albumin, Tf= transferrin, Ova=ovalbumin, MSA=mouse albumin.

Figure 2.

Anti-MDI mAbs recognize MDI-albumin by Western blot. Panel (A) Different mAbs were Western blotted from a native gel (protein stain lanes 1 and 2) against MDI-albumin (odd numbered lanes) or unconjugated albumin (even numbered lanes). Lanes 1, 2 (CE2), lanes 3, 4 (AD3), lanes 5, 6 (DD10), lanes 7, 8 (AH6). *Note all 6 mAbs displayed similar (triple banding) binding patterns. Arrows highlight MDI-albumin monomer, and cross-linked dimers and trimers. Panel (B) Left side shows protein stain of SDS-PAGE, and right side shows Western blot under reducing conditions with mAb BC8. Lanes 1, 5- unconjugated albumin; lanes 2, 6- MDI-albumin prepared with 0.001% (w/v) MDI; lanes 3, 7- MDI-

albumin prepared with 0.01% (w/v) MDI; lanes 4, 8- MDI-albumin prepared with 0.1% (w/v) MDI. *Note all 6 mAbs displayed similar (MDI dose-dependent) binding patterns.

Figure 3. Cross-competition of different mAbs binding to MDI. Competitive inhibition of biotinylated mAb AD3 (panel A) or mAb AH6 (panel B) binding to MDI-albumin by increasing concentrations of unlabeled mAbs as labeled in legend. *Note BC8 and CE2 data are overlapping in panel A.

Table 1

Molecular characteristics of anti-MDI IgG1/kappa mAbs

<i>Heavy Chain</i>	CDR3	#AA IN CDR3	VH	% IDENTITY	J-REGION	% IDENTITY	D-REGION
AD3	AREGRTHFALDY	13	V5-9-2*01	97.22	J4*01	90.74	D1-1*02
AH6	AREGRSFHFAVDY	13	V5-9-2*01	97.22	J4*01	90.74	D5-7*01
DD10	TREGRSFHFAVDY	13	V5-9-2*01	97.22	J4*01	92.59	D5-7*01
BC8	TRGTTTTWPTY	10	V3-2*02	98.91	J3*01	85.42	D1-2*01
CE2	RSFSPYSFEF	10	V6-6*01	97.62	J2*01	93.75	D2-5*01
DA5	TRHPYYRYEGPFAY	14	V5-6-2*01	96.18	J3*01	91.67	D2-14*01
<i>Light Chain</i>	CDR3	#AA IN CDR3	VL	% IDENTITY	J-REGION	% IDENTITY	
AD3	WQGPHEPQT	9	V1-135*01	97.62	J1*01	100.00	
AH6	WQGTTHFPQT	9	V1-135*01	98.64	J1*01	100.00	
DD10	WQGTTHFPQT	9	V1-135*01	99.66	J1*01	100.00	
BC8	QQWSSNPPT	9	V4-59*01	99.66	J5*01	97.14	
CE2	QQWSSYPRT	9	V4-72*01	98.91	J1*01	100.00	
DA5	LQYLSYPLT	9	V9-124*01	98.57	J5*01	94.74	